

**WE CLAIM:**

1. A method for identifying protein-protein interactions comprising prey proteins interacting with one or more bait protein comprising:
  - (a) introducing one or more prey protein in cells, wherein a prey protein is labelled with an epitope tag permitting separation of the prey protein from other proteins in the cells;
  - (b) introducing one or more bait protein in the cells, wherein a bait protein is labelled with a detectable substance permitting detection of the bait protein and protein-protein interactions comprising a prey protein and the bait protein;
  - (c) inducing formation of protein-protein interactions between a prey protein and bait protein; and
  - (d) assaying for protein-protein interactions comprising a prey protein and bait protein by detecting the detectable substance.
2. A method for quantitating protein-protein interactions which method comprises the steps of:
  - (a) introducing one or more prey protein in cells, wherein a prey protein is labelled with an epitope tag permitting separation of the prey protein from other proteins in the cells;
  - (b) introducing one or more bait protein in the cells, wherein a bait protein is labelled with a detectable substance permitting identification of the bait protein and protein-protein interactions comprising a prey protein and the bait protein;
  - (c) inducing formation of protein-protein interactions between a prey protein and bait protein; and
  - (d) quantitating the protein-protein interactions comprising a prey protein and bait protein.
3. A method for quantitating protein-protein interactions which method comprises the steps of:
  - (a) expressing one or more prey protein in cells, wherein a prey protein is labelled with an epitope tag permitting separation of the prey protein from other proteins in the cells;
  - (b) expressing one or more bait protein in the cells wherein a bait protein is labelled with a detectable substance permitting identification of the bait protein and protein-protein interactions comprising a prey protein and the bait protein;
  - (c) obtaining a lysate of the cells and assaying an aliquot of the lysate to measure total expression of the epitope tag and detectable substance;
  - (d) assaying a second aliquot of the lysate to measure the amount of a detectable substance that coprecipitates with an epitope tagged prey protein; and
  - (e) comparing the amounts measured in steps (c) and (d) to quantitate the protein-protein interaction.
4. A method as claimed in claim 3 wherein the cells are subjected to an extracellular or intracellular signal after step (b).
5. A method for determining an interactome for one or more bait protein comprising:
  - (a) preparing recombinant cells each expressing one or more bait protein and one or more prey protein selected from a variegated population of prey proteins;

- (b) inducing formation of protein-protein interactions between a prey protein and bait protein in the cells;
  - (c) identifying protein-protein interactions comprising a prey protein and bait protein to thereby determine the interactome for the bait protein.
- 5 6. A method for determining the function of a gene product comprising:
- (a) defining an interactome of the gene product by preparing recombinant cells expressing the gene product and one or more prey protein selected from a variegated population of prey proteins, and identifying protein-protein interactions comprising the gene product and a prey protein to define the interactome; and
  - 10 (b) determining the function of the gene product based on the structure and/or function of prey proteins that interact with the gene product in the interactome.
7. A method for systematically analyzing protein-protein interactions in cell signalling comprising:
- (a) introducing into cells (i) one or more prey protein labeled with an epitope tag permitting separation of the prey protein from other proteins in the cells; and (ii) one or more bait protein labelled with a detectable substance permitting identification of the bait protein and protein-protein interactions comprising a prey protein and the bait protein;
  - 15 (b) inducing cell signaling in the cells to thereby form protein-protein interactions between a prey protein and bait protein;
  - (c) assaying for protein-protein interactions comprising a prey protein and bait protein at different time points; and
  - 20 (d) comparing the types of protein-protein interactions at the different time points.
8. A method for quantitatively analyzing protein-protein interactions in cell signalling comprising:
- (a) introducing into cells (i) one or more prey protein labeled with an epitope tag permitting separation of the prey protein from other proteins in the cells; and (ii) one or more bait protein labeled with a detectable substance permitting identification of the bait protein and protein-protein interactions comprising a prey protein and the bait protein;
  - 25 (b) inducing cell signaling in the cells to thereby form protein-protein interactions comprising a prey protein and bait protein;
  - (c) quantitating protein-protein interactions comprising a prey protein and bait protein at different time points.
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9. A method for determining changes in an interactome of a mitotic kinase during cell cycle progression comprising:
- (a) introducing into cells (i) one or more prey protein labeled with an epitope tag permitting separation of the prey protein from other proteins in the cells; and (ii) one or more mitotic kinase labelled with a detectable substance permitting identification of the mitotic kinase and protein-protein interactions comprising the mitotic kinase and a prey protein;
  - 35 (b) assaying for protein-protein interactions comprising a prey protein and mitotic kinase at different time points; and
  - (c) comparing the types and kind of protein-protein interactions at the different time points.

10. A method for analyzing protein-protein interactions in different cell types comprising:
- (a) introducing into first cells (i) one or more prey protein labeled with an epitope tag permitting separation of the prey protein from other proteins in the cells; and (ii) one or more bait protein labelled with a detectable substance permitting identification of the bait protein and protein-protein interactions comprising a prey protein and the bait protein;
  - (b) introducing into second cells the same prey protein(s) and bait protein(s) introduced into the first cells in step (a);
  - (c) inducing cell signalling in the cells in (a) and (b) to thereby form in the first and second cells protein-protein interactions comprising a prey protein and bait protein; and
  - (d) comparing the protein-protein interactions identified in the first cells with the protein-protein interactions in the second cells.
11. A method as claimed in claim 11 wherein the first cells are from a subject with a disease and the second cells are normal cells.
12. A method for assaying for changes in protein-protein interactions in response to intracellular or extracellular factors comprising:
- (a) introducing one or more prey protein in cells, wherein a prey protein is labelled with an epitope tag permitting separation of the prey protein from other proteins in the cells;
  - (b) introducing one or more bait protein in the cells, wherein a bait protein is labelled with a detectable substance permitting identification of the bait protein and protein-protein interactions comprising a prey protein and the bait protein;
  - (c) inducing formation of protein-protein interactions between a prey protein and bait protein;
  - (d) introducing an intracellular or extracellular factor;
  - (e) assaying protein-protein interactions comprising a prey protein and bait protein; and
  - (f) comparing the assayed protein-protein interactions with protein-protein interactions assayed in the absence of the intracellular or extracellular factor.
13. A method for identifying a potential modulator of signal transduction activity comprising :
- (a) introducing one or more prey protein in cells, wherein a prey protein is labelled with an epitope tag permitting separation of the prey protein from other proteins in the cell;
  - (b) introducing one or more bait protein in the cells wherein a bait protein is labelled with a detectable substance permitting identification of the bait protein and protein-protein interactions comprising a prey protein and the bait protein;
  - (c) introducing a test agent in the cell;
  - (d) inducing formation of protein-protein interactions between a prey protein and bait protein;
  - (e) assaying protein-protein interactions comprising a prey protein and bait protein; and
  - (f) comparing the protein-protein interactions with the protein-protein interactions obtained in the absence of the test agent to determine the effect of the agent on the protein-protein interactions wherein a change in the protein-protein interactions indicates that the test agent is a potential modulator.

14. A method of claim 13 wherein an increase in the protein-protein interactions indicates that the agent is an agonist of the interaction and a decrease in the amount of protein-protein interactions indicates that the agent is an antagonist.
15. A method of any preceding claim wherein the cells are mammalian cells.
- 5 16. A method as claimed in any preceding claim wherein one bait protein is introduced or expressed in the cells.
17. A method as claimed in any preceding claim wherein two or more bait proteins are introduced or expressed in the cells.
18. A method as claimed in claim 17 wherein each bait protein is labeled with a different detectable substance.
- 10 19. A method as claimed in any preceding claim wherein the detectable substance is an enzyme, radioisotope, fluorescent label, luminescent label, or an enzymatic label.
20. A method of claim 19 wherein the detectable substance is an enzymatic label.
21. A method of claim 20 wherein the detectable substance is luciferase, in particular Renilla luciferase.
- 15 22. A method as claimed in any preceding claim wherein two or more prey proteins are introduced into the cells.
23. A method of any preceding claim wherein the epitope tag is FLAG, hemagglutinin, His6, or an Ig sequence.
24. A method of any preceding claim wherein the prey protein comprises a protein sequence obtained from genomic DNA sequences or random sequences.
- 20 25. A method of any preceding claim wherein the prey protein comprises a library of protein sequences.
26. A method of any preceding claim wherein the bait protein is a functional domain of a protein involved in signal transduction.
27. A method of any preceding claim wherein the bait protein is a protein of the TGF $\beta$  proteome, Wnt/Wingless pathway, Sak/Polo pathway, or a receptor tyrosine kinase pathway.
- 25 28. A method of any preceding claim wherein the bait protein is a Smad protein, SARA family protein, Smad-interacting protein, TGF $\beta$  receptor, TGF $\beta$  receptor interacting protein, SMURF, BMP receptor, APC,  $\beta$ -catenin, axin, dishevelled, GSK-3 $\beta$ , TCFs1-4, Sak, Plks, EGF, FGF, PDGF, or NGF.
- 30 29. A method as claimed in any preceding claim wherein protein-protein interactions are assayed by purifying prey protein and complexes comprising the prey protein based on the epitope tag, and co-purifying the protein-protein interactions comprising the prey protein and bait protein by detecting the detectable substance.
30. A method as claimed in claim 29 wherein the prey protein and complexes are purified by immunoprecipitation with an antibody specific for the epitope tag.
- 35 31. An agent, modulator, or inhibitor identified by a method claimed in any preceding claims.